

Claims:

1. A two-site immunoassay for the qualitative or quantitative detection of alpha-amylase in a test sample, said immunoassay comprising;

(i) exposing said test sample to a first antibody or fragment thereof which specifically or preferentially binds to a first epitope on said alpha-amylase, under conditions permitting binding of said first antibody or fragment thereof to alpha-amylase if present,

(ii) subsequently exposing bound alpha-amylase, if any, to a second antibody or fragment thereof which specifically or preferentially binds to a second epitope on said alpha-amylase that is distinct from said first epitope, under conditions permitting binding of said second antibody or fragment thereof to said bound alpha-amylase, and

(iii) detecting any binding of said second antibody or fragment thereof to said bound alpha-amylase,

wherein either of said first or second epitopes is an epitope comprising one or more of the amino acid sequences; IDRLVSIRTRGQIHS (SEQ ID NO: 1), CRDDRPYADG (SEQ ID NO: 2), VNWVNKVGGS (SEQ ID NO: 3) and variants thereof showing  $\geq 80\%$  sequence identity.

2. An immunoassay according to claim 1, wherein either of said first or second epitopes is an epitope comprising one or more of the amino acid sequences; IDRLVSIRTRGQIHS (SEQ ID NO: 1), CRDDRPYADG (SEQ ID NO: 2), VNWVNKVGGS (SEQ ID NO: 3) and variants thereof showing  $\geq 90\%$  sequence identity.

3. An immunoassay according to claim 1, wherein either of said first or second epitopes is a conformational epitope comprising one or more of the amino acid

sequences; IDRLVSIRTRGQIHS (SEQ ID NO: 1), CRDDRPYADG (SEQ ID NO: 2), VNWVNKVGGS (SEQ ID NO: 3).

4. An immunoassay according to claim 1, wherein either  
5 of said first or second epitopes is a conformational epitope comprising all of the amino acid sequences; IDRLVSIRTRGQIHS (SEQ ID NO: 1), CRDDRPYADG (SEQ ID NO: 2), VNWVNKVGGS (SEQ ID NO: 3).

- 10 <sup>10/22</sup> 5. An immunoassay according to any one of the preceding claims, wherein said first antibody or fragment thereof or said second antibody or fragment thereof is provided bound to a solid support.

- 15 6. An immunoassay according to claim 5, wherein the solid support is selected from microwell plates, membranes, beads, particles, sensors and porous test strips.

- 20 <sup>10/22</sup> 7. An immunoassay according to any one of the preceding claims, wherein binding of the second antibody or fragment thereof to alpha-amylase is detected through the use of a readily detectable label.

- 25 8. An immunoassay according to claim 7, wherein the detectable label is selected from detectable enzymes, radioisotopes, luminescent labels and fluorescent labels.

- 30 <sup>10/22</sup> 9. An immunoassay according to any one of claims 1 to 6, wherein binding of the second antibody or fragment thereof to alpha-amylase is detected through the use of immunochromatography or agglutination.

- 35 10. An immunoassay according to any one of the preceding claims, wherein at least one of the first and second antibodies or fragments thereof is selected from

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monoclonal antibodies or fragments thereof and recombinant antibody fragments.

11. An immunoassay according to any one of the preceding claims, wherein the test sample is obtained from a cereal grain.

12. An immunoassay according to claim 11, wherein the cereal grain is selected from the group consisting of bread wheat (*Triticum aestivum*), durum wheat (*Triticum turgidum* var. durum), club wheat (*Triticum compactus*), rye (*Secale cereale*), triticale (*Triticosecale* species) and barley (*Hordeum vulgare*).

13. An immunoassay according to claim 11 or 12, wherein the test sample is an aqueous extract from grain, grain meal or flour.

14. An immunoassay according to any one of the preceding claims, wherein said immunoassay provides for the quantitative detection of alpha-amylase by further comprising;

(iv) comparing the level of detected binding of the second antibody or fragment thereof to alpha-amylase against a suitable standard.

15. An immunoassay according to any one of the preceding claims when used to detect weather damage in a cereal grain.

16. A monoclonal antibody or fragment thereof, recombinant antibody or fragment thereof, recombinant antibody fragment or binding partner which specifically or preferentially binds to an epitope on alpha-amylase comprising one or more of the amino acid sequences; IDRLVSIIRTRGQIHS (SEQ ID NO: 1), CRDDRPYADG (SEQ ID NO: 2),

VNWNKVGGS (SEQ ID NO: 3) and variants thereof showing  $\geq$  80% sequence identity.

5 17. A monoclonal antibody or fragment thereof,  
recombinant antibody or fragment thereof, recombinant  
antibody fragment or binding partner which specifically  
binds to an epitope on alpha-amylase comprising one or  
more of the amino acid sequences; IDRLVSIRTRGQIHS (SEQ ID  
NO: 1), CRDDRPYADG (SEQ ID NO: 2), VNWNKVGGS (SEQ ID NO:  
10 3) and variants thereof showing  $\geq$  90% sequence identity.

15 18. A monoclonal antibody or fragment thereof,  
recombinant antibody or fragment thereof, recombinant  
antibody fragment or binding partner which specifically or  
preferentially binds to a conformational epitope on alpha-  
amylase comprising one or more of the amino acid  
sequences; IDRLVSIRTRGQIHS (SEQ ID NO: 1), CRDDRPYADG (SEQ  
ID NO: 2), VNWNKVGGS (SEQ ID NO: 3).

20 19. A monoclonal antibody or fragment thereof,  
recombinant antibody or fragment thereof, recombinant  
antibody fragment or binding partner which specifically or  
preferentially binds to conformational epitope on alpha-  
amylase comprising all of the amino acid sequences;  
25 IDRLVSIRTRGQIHS (SEQ ID NO: 1), CRDDRPYADG (SEQ ID NO: 2),  
VNWNKVGGS (SEQ ID NO: 3).

30 20. A kit for performing a two-site immunoassay for the  
qualitative or quantitative detection of alpha-amylase in  
a test sample, said kit comprising a container or solid  
support including a monoclonal antibody or fragment  
thereof, recombinant antibody or fragment thereof,  
recombinant antibody fragment or binding partner according  
to any one of claims 16 to 19.

21. A kit according to claim 20, further comprising a container including an aqueous extraction agent for extracting alpha-amylase from grain, grain meal or flour.
- 5 22. A kit according to claim 20, wherein the extraction agent is aqueous NaCl or CaCl<sub>2</sub>.

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